

A Quantitative Description of Suicide Inhibition of Dichloroacetic Acid in Rats and Mice

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Dichloroacetic acid (DCA), a minor metabolite of trichloroethylene (TCE) and water disinfection byproduct, remains an important risk assessment issue because of its carcinogenic potency. DCA has been shown to inhibit its own metabolism by irreversibly inactivating glutathione transferase zeta (GSTzeta). To better predict internal dosimetry of DCA, a physiologically based pharmacokinetic (PBPK) model of DCA was developed. Suicide inhibition was described dynamically by varying the rate of maximal GSTzeta-mediated metabolism of DCA (V_{\max}) over time. Resynthesis (zero-order) and degradation (first-order) of metabolic activity were described. Published iv pharmacokinetic studies in naïve rats were used to estimate an initial V_{\max} value, with K_m set to an *in vitro* determined value. Degradation and resynthesis rates were set to estimated values from a published immunoreactive GSTzeta protein time course. The first-order inhibition rate, k_d , was estimated to this same time course. A secondary, linear non-GSTzeta-mediated metabolic pathway is proposed to fit DCA time courses following treatment with DCA in drinking water. The PBPK model predictions were validated by comparing predicted DCA concentrations to measured concentrations in published studies of rats pretreated with DCA following iv exposure to 0.05 to 20 mg/kg DCA. The same model structure was parameterized to simulate DCA time courses following iv exposure in naïve and pretreated mice. Blood and liver concentrations during and postexposure to DCA in drinking water were predicted. Comparisons of PBPK model predicted to measured values were favorable, lending support for the further development of this model for application to DCA or TCE human health risk assessment.

Key Words: (3–6) DCA; PBPK; GSTzeta; suicide inhibition; rat; mouse.

This research has not been subjected to required peer and policy review by either the Department of Energy or the United States Environmental Protection Agency and, therefore, does not necessarily reflect the views of these agencies, and no official endorsement should be inferred. The animal use described in these studies was conducted in accordance with the principles stated in the “Guide for the Care and Use of Laboratory Animals,” National Research Council, 1996, and the Animal Welfare Act of 1996, as amended.

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Dichloroacetic acid (DCA) is a minor metabolite of trichloroethylene (TCE), a ubiquitous air and water contaminant which is used in dry cleaning, for metal degreasing, and as a general-purpose solvent. DCA is also a by-product of chlorination and occurs at low levels in most drinking water systems disinfected with chlorine. In addition, DCA has been used clinically for over 25 years to treat several metabolic disorders. Currently the most active pharmaceutical use is for the treatment of congenital lactic acidosis (U.S. EPA, 2003).

DCA has been proposed as one of the key metabolites responsible for TCE-induced liver carcinogenicity in mice (Bull, 2000). DCA causes a variety of adverse effects in humans and animals. Effects of DCA treatment in humans have been limited to transient central neuropathy, peripheral neuropathy (tingling in fingers and toes and nerve conduction changes), and metabolic changes (U.S. EPA, 2003). Noncancer effects in animals include metabolic effects (Davis, 1990; Evans and Stacpoole, 1982), hepatic toxicity (Bhat *et al.*, 1991; Sanchez and Bull, 1990), reproductive/developmental toxicity (Linder *et al.*, 1997; Smith *et al.*, 1992; Toth *et al.*, 1992), and neurotoxicity (Moser *et al.*, 1999). DCA has been shown to increase both incidence and multiplicity of hepatic adenomas and adenocarcinomas in male (Bull *et al.*, 1990; Daniel *et al.*, 1992; DeAngelo *et al.*, 1991, 1999; Ferreira-Gonzalez *et al.*, 1995; Herren-Freund *et al.*, 1987) and female B6C3F₁ mice (Pereira, 1996; Pereira and Phelps, 1996; U.S. EPA, 1991) and in male F344 rats (DeAngelo *et al.*, 1996; Richmond *et al.*, 1995).

Pharmacokinetic studies of DCA have been performed in mice (e.g., Gonzalez-Leon, *et al.*, 1999; Schultz *et al.*, 2002), rats (e.g., Gonzalez-Leon *et al.*, 1997; Saghir and Schultz, 2002) and humans (e.g., Curry *et al.*, 1985, 1991). Metabolic clearance of DCA from the blood is very rapid. Pharmacokinetic differences exist between routes of DCA intake, with concentration-time profiles in rats exhibiting secondary peaks of DCA after oral gavage dosing (Saghir and Schultz, 2002). In animal studies, only a small fraction of DCA is found in feces and urine, although the amount of DCA in urine was reported to increase with DCA dose (Lin *et al.*, 1993).

The primary metabolic pathway for DCA biotransformation is glutathione-dependent oxidative dechlorination of DCA to

glyoxylate which is catalyzed by glutathione transferase zeta (GSTZ1-1; Tong *et al.*, 1998b). The existence of a second metabolic pathway for DCA involving reductive dechlorination to monochloroacetic acid (MCA) has been proposed previously (Larson and Bull, 1992; Stacpoole *et al.*, 1990). However, Ammini and colleagues (2003) reported finding no appreciable MCA in the urine of GSTzeta knockout mice exposed to 50 mg/kg DCA by oral gavage. Alternatively, kinetic studies in male rats using the chiral dihaloacetates, bromochloro and fluorochloroacetate (BCA, FCA), provide indirect evidence that di-haloacetates are metabolized by an additional glutathione-dependent pathway that also forms glyoxylate (Schultz and Sylvester, 2001). Specifically, the initial rate of metabolism for the (+) BCA stereoisomer is not altered after loss of GSTzeta activity due to prior DCA exposure, which is in contrast to (–) BCA and (–, +) FCA (Schultz and Sylvester, 2001). Based on the (–, +) BCA data, this additional pathway was estimated to account for less than 20% of the overall metabolic clearance for di-haloacetates. Further information regarding the specific mechanism(s) for this pathway remain to be elucidated.

Interestingly, DCA has been shown to inhibit its own metabolism in rodents (Gonzalez-Leon *et al.*, 1997, 1999; Schultz *et al.*, 2002) and humans (Curry *et al.*, 1991). The inhibition has been proposed to occur from the formation of a covalent bond between GSH and DCA which results in a *S*-(α -chlorocarboxymethyl)glutathione intermediate (Anderson *et al.*, 1999; Wempe *et al.*, 1999). This intermediate then gives rise to a carbonium \leftrightarrow sulfonium ion that either undergoes hydrolysis to form glyoxylate, regenerating GSH, or alternatively, reacts with GSTzeta to covalently modify and inactivate the enzyme (Tzeng *et al.*, 2000). Although prolonged exposure to DCA greatly decreases its metabolism, biotransformation is still the primary pathway for DCA elimination (Gonzalez-Leon *et al.*, 1997, Saghir and Schultz, 2002), which is also consistent with the existence of a secondary metabolic pathway that is not altered by loss of GSTzeta activity.

Barton and colleagues (1999) developed a simple physiologically based pharmacokinetic (PBPK) model to describe blood concentrations of DCA in mice. This model consists of three compartments: body, liver, and gastrointestinal tract lumen and describes administration of DCA via iv injection, oral gavage, or by drinking water and includes both urinary and metabolic clearance of DCA. Suicide inhibition of DCA metabolism was described empirically as a function of ingestion rates. The transition from uninhibited to inhibited metabolism was assumed to occur linearly over the drinking water concentrations of 0.02–1.0 g/l given for 2 weeks. In this model, metabolism was not completely inhibited at concentrations >1 g/l.

Abbas and Fisher (1997) included a submodel of DCA in their PBPK model describing TCE absorption, metabolism, and clearance in mice. The DCA submodel tracks metabolically derived DCA in lung, body, kidney, and liver compartments. The sole source of clearance of DCA was metabolism, which was described as a saturable process.

The Abbas and Fisher DCA PBPK model did not include a description of suicide inhibition of DCA. The empirical nature of the description of suicide inhibition in the Barton PBPK model limits its predictive ability under conditions of partial inhibition, varying drinking water scenarios (e.g., longer or shorter exposure period, longer or shorter recovery time), or other routes of exposure (e.g., suicide inhibition due to prior bolus exposure).

Our objective was to develop a PBPK model for DCA in rodents, which includes a description for inactivation of GSTzeta and corresponding inhibition in the metabolism of DCA. Our approach was to utilize the wide array of both published and unpublished pharmacokinetic datasets for DCA focusing exclusively on intravenous and drinking water routes of exposure. Sole use of intravenous pharmacokinetic studies obviates the need for more complicated descriptions of DCA oral absorption kinetics and is more applicable to describing metabolically derived DCA from TCE. Drinking water is the major route of exposure for the general population to DCA (U.S. EPA, 2003). Eventually the DCA PBPK model is expected to help address the risks posed from ingestion of DCA-contaminated drinking water directly or from ingestion of chlorinated solvents that are expected to be metabolized to DCA, such as TCE.

MATERIALS AND METHODS

Animals and drinking water. Male B6C3F1 mice and Fischer 344 rats (Charles Rivers Laboratories, Raleigh, NC) were maintained in polycarbonate cages with hardwood chip bedding. All were housed one per cage. Food and water were available *ad libitum*.

Chemicals. All chemicals were purchased from Sigma Chemical Company.

Drinking water experiment. Treated mice ($n = 5/\text{time point}$) were provided with drinking water containing 2.0 g/l of DCA neutralized to pH 7 with NaOH. Treated rats ($n = 5/\text{time point}$) were provided with drinking water containing 0.5 or 2.0 g/l DCA neutralized to pH 7 with NaOH. An aliquot of drinking water solution was sampled daily for the duration of the study and analyzed by gas chromatography to assure the DCA solution was stable and the concentration was accurate. Control rats and mice were placed on drinking water containing sodium chloride at the same concentration as the dose group. Drinking water was contained in 60-ml glass tubes capped with rubber stoppers. The drinking tubes were designed to prevent dripping. Water bottles were weighed daily to determine volume of consumption and dose rate. After 14 days rats and mice were taken off treated drinking water and given pure water. Mice were sacrificed by CO₂ asphyxiation at various time points up to 8 h posttreatment. Blood samples were collected via the inferior vena cava, and whole livers were removed. Rats were bled at various time points up to 8 h via the lateral tail vein using a 25 gauge needle fitted with a heparinized capillary tube. At the last time point the rats were sacrificed by CO₂ inhalation, and blood and liver were collected as with the mice. For all animals a 200-mg portion of the liver was removed for analysis.

Sample preparation and analysis. 200 ml of blood were mixed with 20% lead acetate to quench any metabolism (Ketcha *et al.*, 1996) and stored at –80°C. 200-mg liver samples were quenched with 400 ml of 20% lead acetate, homogenized, and stored at –80°C pending analysis. DCA was esterified and quantified in the blood and liver samples by gas chromatography (Abbas and Fisher, 1997).

IV dosing experiment. DCA blood concentrations following an iv dose of 5 mg/kg DCA to pretreated (2 g/l DCA in drinking water for 14 days) mice

were collected and used for validation of the mouse model. Methodological details are the same as described by Schultz and colleagues (2002).

Published data. Original data files (Saghir and Schultz, 2002) containing DCA plasma time courses in naïve and DCA pretreated (0.2 g/l in drinking water for 7 days) rats following iv exposures ranging from 0.05 to 20 mg/kg were used for rat model development and validation. Plasma and whole blood were used interchangeably based on the blood/plasma ratio of 0.91 for DCA measured by Schultz and colleagues for rats (1999). A time course for inhibition of immunoreactive GSTzeta protein levels in rats following ip exposure to 38.7 mg/kg DCA was digitized from the work of Anderson and colleagues (1999) using Grab It! (Datatrend Software, Raleigh, NC) and used in model development.

Original data files for DCA whole blood time courses in naïve and pretreated (2 g/l DCA in drinking water for 14 days) mice following iv exposures to 20 mg/kg and recovery times ranging from 6 to 48 h collected by Schultz and colleagues (2002) were utilized for mouse model development. DCA blood and liver time courses in mice during exposure to DCA at 2 g/l in drinking water measured by Schultz and colleagues (2002) were also used. Please consult the respective original publications for methodological details.

Statistics. Daily water consumption amounts were analyzed for statistical differences using Analysis of Variance (ANOVA), $\alpha = 0.05$. Multiple comparisons were performed using least significant differences, $\alpha = 0.05$.

PBPK model structure. Figure 1 shows the basic structure of the DCA PBPK model. DCA was tracked in kidney, liver, slowly and rapidly perfused tissues. Rapid venous equilibration was assumed for the blood. IV dosing was described as an infusion directly into the blood. Ingested DCA in drinking water was delivered directly into the liver. The rate of DCA dosing (mg/h) was calculated by multiplying the drinking water concentration (mg/ml) by the rate of drinking water consumption (ml/h). The time-dependent rate of drinking water consumption was calculated by multiplying the average daily water consumption (ml) by the time-dependent percentage of total daily water intake (1/h). Water intake percentages in 0.5-h intervals were obtained for female B6C3F1 mice from the publication of Yuan (1993). Scaled average daily water consumption rates in Table 1 were calculated by dividing measured average daily water consumption levels presented in Table 2 by the $3/4$ power of mean body weights. Since average daily water consumption was significantly reduced in rats exposed to 2 g/l and 0.5 g/l DCA in drinking water, dose-specific water consumption values were used to calculate a scaled average daily water consumption rate for rats exposed to 0.5 or 2 g/l DCA. DCA clearance was attributed solely to metabolism. Urinary clearance of DCA was not included, as only a small fraction (0.6%) of total DCA doses has been measured unchanged in the urine following a 28.2 mg/kg oral gavage dose of DCA (Lin *et al.*, 1993). However, for modeling higher doses of DCA, urinary excretion should be considered.

Metabolism of DCA was modeled with two alternative descriptions. The first description included the GSTzeta-mediated pathway only, while the second description included two pathways, one GSTzeta-mediated pathway representing the oxidation of DCA to glyoxylic acid and a second linear proposed pathway representing a secondary, nonspecific pathway of DCA metabolism. Thus, the rate of DCA metabolism in the liver was described as

$$\frac{dA_M}{dt} = \frac{V_{\max} \cdot C_{vl}}{K_m + C_{vl}} + k_f \cdot A_l,$$

where K_m is the Michaelis-Menten affinity constant (mg/l), k_f is the linear rate of metabolism (1/h), C_{vl} is the concentration of DCA in the venous blood of the liver (mg/l), and A_l is the amount of DCA in the liver (mg). For the first alternative model, k_f was set to zero to describe GSTzeta-mediated metabolism only. Suicide inhibition of DCA metabolism was described dynamically by having the rate of maximal inhibitable metabolism of DCA (V_{\max}) decrease proportional to the rate of GSTzeta-mediated DCA metabolism. This reflects the current proposed mechanism of DCA-dependent inactivation of DCA metabolism via inactivation of GSTzeta. Anderson and colleagues (1999) propose that a short-lived, glutathione-bound intermediate of DCA metabolism reacts with GSTzeta to yield covalently modified GSTzeta. For short-lived intermediates, the rate of metabolism has been used as a surrogate measure of intermediate liver concentration

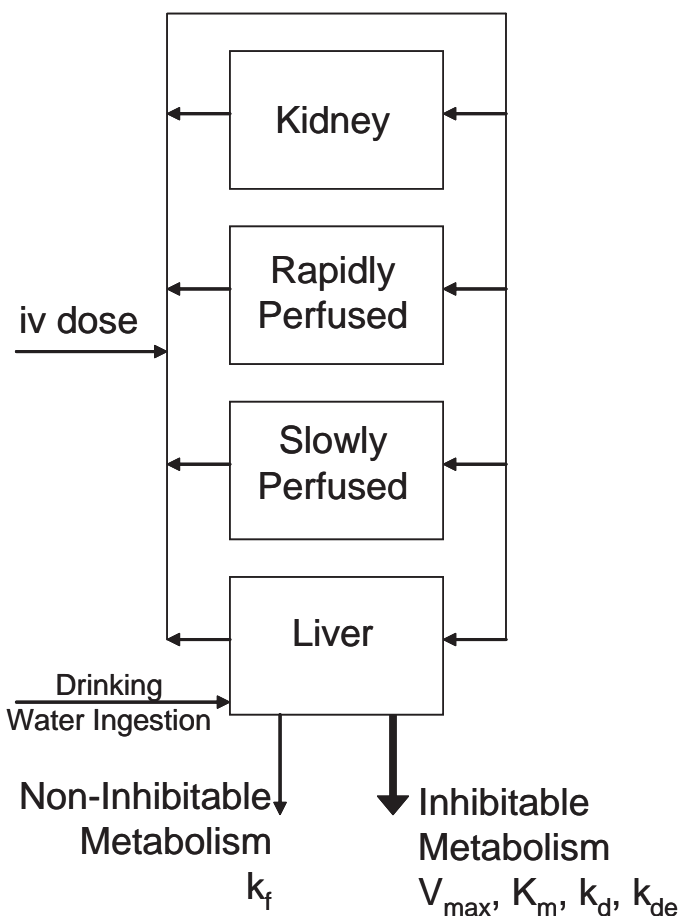


FIG. 1. Schematic of DCA PBPK Model. DCA is tracked in four compartments (kidney, liver, rapidly and slowly perfused tissues). Administration of DCA by iv injection or drinking water is described. DCA is eliminated by metabolism in the liver. Suicide inhibition of DCA is described (see text for details).

(Lilly *et al.*, 1998). Resynthesis (zero-order) and degradation (first-order) of metabolic activity were also described by incorporating resynthesis and degradation of V_{\max} . The rate of change of V_{\max} is described by

$$\frac{dV_{\max}}{dt} = -k_d \cdot \frac{V_{\max} \cdot C_{vl}}{C_{vl} + K_m} + k_s - k_{de} \cdot V_{\max}$$

and

$$V_{\max} = \int_0^t \frac{dV_{\max}}{dt} + V_{\max 0}$$

where k_d is the inhibition constant (1/h), k_s is the rate of resynthesis (mg/h²), k_{de} is the degradation rate (1/h), and $V_{\max 0}$ is the initial value for V_{\max} (mg/h). To maintain equilibrium of enzyme at steady state, $k_s = k_{de} \cdot V_{\max 0}$ and k_f were allometrically scaled to the $3/4$ and $-1/4$ powers of body weight, respectively.

Fixed model parameters. Species-specific physiological parameters, displayed in Table 1, were taken from the work of Brown and colleagues (1997) with the exception of cardiac output rates, which were taken from the work of Arms and Travis (1988). DCA tissue:blood partition coefficients as determined by Abbas and Fisher (1997) in B6C3F1 mice and shown in Table 3 were used for both mice and rats. The Michaelis-Menten affinity constant, K_m , was set to the value estimated by Tong and colleagues (1998a) using an *in vitro* liver cytosol enzyme-activity assay. The degradation rate was set at a value estimated

TABLE 1
Physiological and Drinking Water Parameters

Description	Rat	Mouse	Source
Body weight (BW) (kg)	0.189–0.305	0.021–0.028	Experiment
Cardiac output (Q_{CC}) (l/h/kg ^{3/4})	14.1	16.5	Arms and Travis, 1988
<i>Blood flow fraction of cardiac output (%)</i>			
Liver (Q_{LC})	18.3	16.1	Brown <i>et al.</i> , 1997
Kidney (Q_{KC})	14.1	9.1	Brown <i>et al.</i> , 1997
Slowly perfused tissues (Q_{SC})	41	41	Brown <i>et al.</i> , 1997
Rapidly perfused tissues (Q_{RC})	26.6	33.9	59% – Q_{LC} – Q_{KC}
<i>Compartment volumes fraction of body weight (%)</i>			
Liver (V_{LC})	3.66	5.49	Brown <i>et al.</i> , 1997
Kidney (V_{KC})	0.73	1.67	Brown <i>et al.</i> , 1997
Slowly perfused tissues (V_{SC})	62	62	Brown <i>et al.</i> , 1997
Rapidly perfused tissues (V_{RC})	15.61	12.83	20% – V_{LC} – V_{KC}
<i>Water consumption rates (ml/kg^{3/4})</i>			
0.2 g/l DCA (Drink _C)	56.1 ^a	61.4 ^b	Calculated
0.5 g/l DCA (Drink _C)	54.4	—	Calculated
2 g/l DCA (Drink _C)	43.2	61.4 ^b	Calculated

^aBased on control rat water consumption.

^bBased on control mouse water consumption.

TABLE 2
Daily Water Consumption for control and treated rodents

Concentration of DCA in Drinking Water (g/l)	Mouse (ml)	Rat (ml)
0	4.2 ± 0.4 (n = 9)	22.7 ± 1.5 (n = 8)
0.5		19.9 ^a ± 2.1 (n = 5)
2	4.1 ± 0.4 (n = 32)	15.0 ^a ± 1.8 (n = 5)

^aSignificantly different than controls ($p < 0.05$).

TABLE 3
DCA Partition Coefficients

Description	Value	Source
Liver/blood	1.08	Abbas and Fisher, 1987
Rapidly perfused/blood	1.08	Assumed equal to liver
Slowly perfused/blood	0.37	Abbas and Fisher, 1987
Kidney/blood	0.14	Abbas and Fisher, 1987

previously from an immunoreactive GSTzeta protein time course following iv exposure to 38.7 mg/kg DCA (Anderson *et al.*, 1999).

Adjustable model parameters. Parameter estimation followed a stepwise methodology, where a single parameter was fit to one dataset at a time to obtain

intermediate parameter estimates, designed in such a way that each parameter was fit to the dataset which the parameter was judged to be most sensitive. Then a global parameter estimation was performed where all parameters were fit to all the datasets to obtain final parameter estimates.

IV plasma time courses in naïve rats (Saghir and Schultz, 2002) following administration of 1, 5, or 20 mg/kg DCA were used to estimate an intermediate V_{maxOC} value. The first-order inhibition rate, k_d , was set to an initial estimate of 2.1 h⁻¹, and the linear metabolism rate, k_{fc} , was set to 0 h⁻¹ for alternative model 1. The first-order inhibition rate, k_d , was then fit to an immunoreactive GSTzeta protein time course (Anderson *et al.*, 1999) with V_{maxOC} at its intermediate estimated value and k_{fc} set again to 0 h⁻¹. Next, DCA blood time courses following 14 days exposure to 0.5 and 2 g/l DCA in drinking water were simulated with both V_{maxOC} and k_d set to their intermediate estimated values. Finally, a global parameter estimation, using intermediate estimates and alternate values as starting values, was run fitting both V_{maxOC} and k_d to all datasets (three naïve rat time courses, one protein time course, two drinking water time courses). Parameters were estimated by maximum likelihood techniques as implemented in ACSL Math V 2.5.4 (Aegis Technologies, Huntsville, AL). The Nelder-Mead algorithm was used for likelihood estimation. The error model was set to relative. Three different starting values that resulted in successful conclusion of the Nelder-Mead algorithm were used for each adjustable parameter.

For alternative model 2, the same parameter estimation methodology was performed, with the exception that the linear metabolism rate, k_{fc} , was set to an initial estimate of 1.05 h⁻¹ during stepwise estimation of V_{maxOC} and k_{fc} . The linear metabolism rate, k_{fc} , was fit to the DCA blood time course following 14 days exposure to 0.5 and 2 g/l DCA in drinking water with both V_{maxOC} and k_{fc} set to their intermediate estimated values. A global parameter estimation, using intermediate estimates and alternate values as starting values, was run fitting all three estimated model parameters (V_{maxOC} , k_d , k_{fc}) to all datasets (three naïve rat time courses, one protein time course, two drinking water time courses).

A similar fitting methodology was attempted to parameterize the mouse PBPK model. Instabilities in the log-likelihood function used for parameter estimation in ACSL Math resulted in parameter estimates that were irreproducible from varying starting values and judged inferior to initial and final visual estimates. Thus, the decision was made to fit parameters visually rather than by maximum likelihood. For models 1 and 2, a value for V_{maxOC} was fit to an iv time course in naïve mice (20 mg/kg DCA) (Schultz *et al.*, 2002). The degradation rate for the mouse was kept at the value estimated from the rat GSTzeta protein time course. The first-order inhibition rate was then fit to time courses in mice following administration of 20 mg/kg with recovery times ranging from 6 to 48 h (Schultz *et al.*, 2002). Subsequently, for model 2, the linear metabolism rate, k_{fc} , was fit to the DCA blood time courses from mice previously treated for 14 days with 2 g/l DCA in drinking water that either continued (Schultz, *et al.*, 2002) or were removed from treatment (current study). This parameter estimation sequence was iterated until no further adjustment of model parameters was deemed necessary for visual agreement (two iterations total). Final parameter estimates for metabolic and suicide inhibition parameters are given in Table 4.

Model discrimination. Model discrimination between alternative nested models 1 and 2 was performed by the log-likelihood ratio test (Andersen *et al.*, 2001; Collins *et al.*, 1999; Keys *et al.*, 1999). Plasma DCA concentration and percentage reduction in V_{max} predictions were compared between alternative models 1 and 2 in the rat.

Validation. The calibrated rat model, was used to simulate blood concentrations of DCA in pretreated (0.2 g/l in drinking water for 7 days) rats following iv doses ranging from 0.05 to 20 mg/kg DCA and compared to time courses collected by Saghir and Schultz (2002) for validation purposes. The mouse model, using visual parameter estimates, was used to simulate blood concentrations of DCA in pretreated (2 g/l in drinking water for 14 days) mice following an iv dose of 5 mg/kg DCA and compared to measured values from the current study for validation.

The PBPK model was also used to simulate liver concentrations in rats after 14 days exposure to 0.5 or 2 g/l DCA and in mice after 14 days exposure to 2 g/l DCA. The simulation results were compared to measured time points from the current study to assess the model's ability to predict liver concentrations in addition to blood.

TABLE 4
Metabolic and Suicide Inhibition Parameters

Description	Units	Rat ^a	Mouse
Initial rate of maximal inhibitable metabolism ($V_{\max 0C}$)	mg/h/kg ^{3/4}	77.5 ^b	190 ^c
Michaelis-Menten affinity constant (K_m)	Mg/L	9.0 ^d	10.6 ^d
Linear metabolism rate (k_{fC})	1/h	1.2 ^b	1.0 ^c
Inhibition constant (k_d)	1/h	2.6 ^b	0.6 ^c
Degradation rate (k_{dc})	1/h	0.00875 ^e	0.00875 ^e

^aFit to alternative model 2.

^bFit by maximum likelihood estimation to rat time-course data.

^cFit visually to mouse time-course data.

^dTong *et al.*, 1998a.

^eAnderson *et al.*, 1999.

Simulations of the rates of metabolism for both metabolic pathways. The PBPK model was used to simulate 14 days pretreatment with drinking water at 0, 0.2, or 2 g/l in rats or mice with a 16-h recovery period followed by a 20-mg/kg iv dose. Control water consumption rates were assumed for both rats and mice at the DCA 0.2 g/l concentration, and for mice at the 2 g/l DCA concentration. The calculated 2 g/l DCA water consumption rate based on the current study was used for rats at the 2 g/l concentration to reflect the decreased drinking behavior of rats at this concentration. The total rate of metabolism and the individual rates of metabolism for the GSTzeta-mediated (pathway 1) and secondary, nonspecific (pathway 2) pathways were recorded at 3 min post iv exposure. Simulations predicted that 3 min post exposure was when the rate of total metabolism was at a maximum.

RESULTS

Drinking Water Consumption

Daily water consumption measurements for control and treated mice and rats are shown in Table 2. Daily water consumption for treated mice was not different from control mice. For rats, daily water consumption was significantly reduced at both the 2 and 0.5 g/l DCA doses compared to controls.

Model Discrimination

Alternative model 2 which included a secondary metabolic pathway gave a significantly better description of DCA plasma levels and percent reduction in GSTzeta protein levels in the rat ($p < 0.005$). Parameter estimates for $V_{\max 0C}$ (79.1 mg/h/kg^{3/4}, model 1; 77.5 mg/h/kg^{3/4}, model 2) and k_d (2.45 1/h, model 1; 2.48 1/h, model 2) were quite similar. Alternative model 2 clearly improved descriptions of DCA plasma levels in rats following DCA treatment in drinking water. Accumulation of DCA in the plasma was predicted by alternative model 1, which was not seen in the data. Accumulation of DCA in the plasma was also predicted by alternative model 1 in the mouse, but this was also not seen in the data. All subsequent results discussed and presented below are from alternative model 2, with secondary DCA metabolism unless otherwise noted.

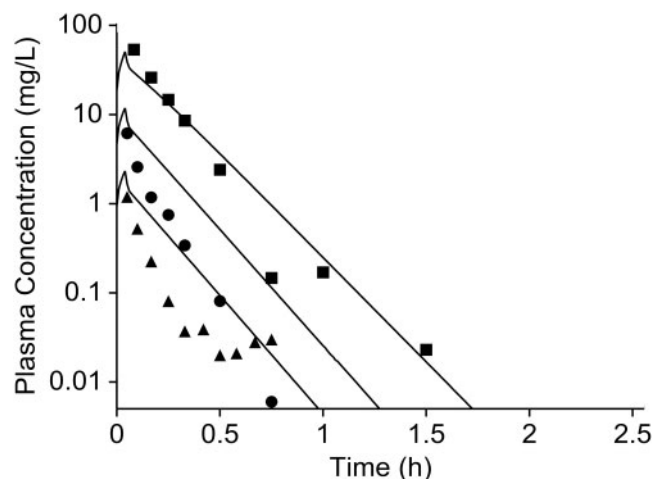


FIG. 2. Model simulations vs. measured concentrations of DCA in the blood of naïve rats exposed to 1 (▲), 5 (●), or 20 (■) mg/kg DCA via iv injection. Measured values originally published by Saghir and Schultz (2002) and are presented as mean values ($n = 4-6$).

Rat IV Simulations

Initial calibration of the initial maximal rate for GSTzeta-mediated metabolism, $V_{\max 0C}$, to naïve rat plasma data shown in Figure 2 yielded an estimate of 71.7 mg/h/kg^{0.75}. For this concentration range (1 to 20 mg/kg), simulations were insensitive to subsequent changes in the inhibition constant, k_d . The final parameter estimate, 77.5 mg/h/kg^{0.75}, obtained from simultaneous estimation of $V_{\max 0C}$, k_d , and k_{fC} was somewhat larger than the initial estimate given above. Plasma DCA concentrations were well predicted following iv administration of 20 mg/kg as shown in Figure 2. However, clearance of DCA from the plasma at the 1- and 5-mg/kg doses was underpredicted.

The results of Anderson and colleagues as shown in Figure 3 indicate 7 days were necessary for complete recovery of GSTzeta enzyme levels from a 38.7-mg/kg DCA ip infusion in the rat. Following adjustment of the inhibition constant, k_d , model simulations of V_{\max} compared favorably to the measured GSTzeta protein time course when both were expressed as percentage reduction. The simulation predicted a faster initial decrease and slower resynthesis of enzyme than measured, which may indicate a time lag in enzyme production compared to model predictions that are based on an instantaneous relationship between metabolism rate and V_{\max} . The initial estimate of k_d was 1.6 h⁻¹, which was increased to 2.5 h⁻¹ in the simultaneous parameter estimation.

Rat Drinking Water Simulations

The addition of noninhibited metabolism was necessary to simulate blood concentrations of DCA in rats following 14 days of treatment with 2 or 0.5 g/l DCA in their drinking water (Fig. 4a). The model clearly overpredicted the measured blood levels without the addition of the secondary metabolic

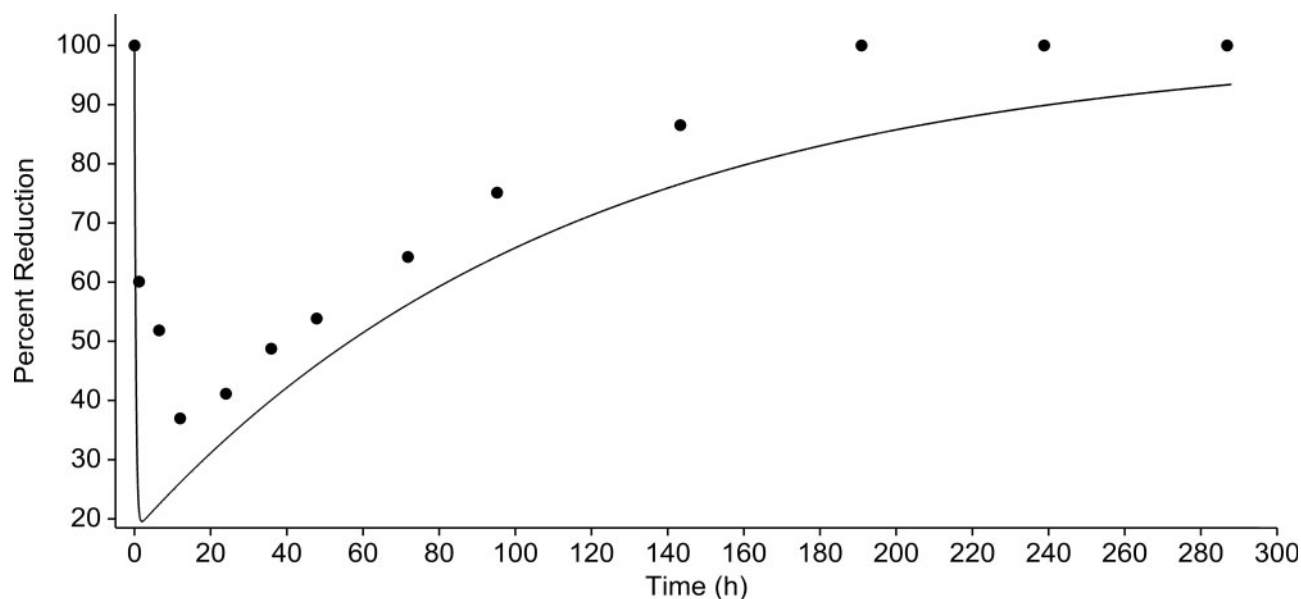


FIG. 3. Model simulations of the percentage reduction of the time-dependent maximum rate of DCA metabolism, $V_{\max}(t)$ from initial $V_{\max 0}$ compared to measured percent of control immunoreactive GSTzeta protein levels (●) following an ip infusion of 38.7 mg/kg DCA. Measured values were originally published by Anderson and colleagues (1999) and are presented as mean values ($n = 3$).

pathway. After adjustment of k_{fC} , initially to 0.90 h^{-1} and finally to 1.15 h^{-1} , clearance of DCA from the blood at both drinking water concentrations was slightly overpredicted. Both the full 15-day span (Fig. 4b) and the 8-h postexposure period (Fig. 4c) are shown.

Rat Model Validation

Simulations to predict the pharmacokinetic behavior of DCA in rats were performed without further adjustment of model parameters (Fig. 5). Validation of model predictions with plasma DCA concentration time courses in pretreated rats exposed to a wide range (0.05–20 mg/kg) of DCA by iv infusion agreed, for the most part, with measured plasma DCA concentrations. Predictions were best with 0.05-, 5-, and 20-mg/kg dose groups, while clearance was somewhat underpredicted following administration of 1 and 0.25 mg/kg DCA. Thus, there seemed to be no systematic bias (consistently better at lower or higher exposures) in model predictions.

Mouse IV Simulations

The time course for DCA in blood of naïve mice treated with 20 mg/kg DCA is biphasic, with an initial more rapid rate of metabolism which slowed after 30 min (Fig. 6). The model, however, could not replicate this biphasic pattern, and the decision was made to concentrate fitting efforts on the earlier portion of the curve to enable the model to provide a better quantitative prediction of DCA blood area-under-the-curve. $V_{\max 0C}$ was visually fit to be $190 \text{ mg/h/kg}^{0.75}$. Higher values, up to

$2000 \text{ mg/h/kg}^{0.75}$, slightly improved model fit but were biologically unrealistic.

In Figure 7, model predictions of DCA blood concentrations in mice pretreated with 2 g/l DCA for 14 days, followed by a range of recovery periods (6–48 h) before dosing intravenously with 20 mg/kg iv dose are shown. The differences between time courses with differing recovery periods would be expected to be due to different amounts of resynthesis of GSTzeta enzyme. The biphasic behavior seen in the control mice time course was not seen in the pretreated mice time courses with the exception of the 48-h recovery period time course which showed a slight indication of decreased clearance at 30 min postexposure. The GSTzeta resynthesis rate for mice was kept at the value calculated for rats. A k_d value of 0.6 h^{-1} gave the best fits taking into account all recovery times.

Mouse Drinking Water Simulations

The predicted clearance of DCA from the blood in mice previously treated for 14 days with 2 g/l DCA in drinking water versus measured values is shown in Figure 8 from models without and with the minor, secondary pathway of DCA metabolism. The model without (Fig. 8a) the minor pathway clearly overpredicts the measured values, while the predictions from the model with the minor pathway (Fig. 8b,c) match the measured values much better. Also shown (Fig. 8d) are model (with the secondary pathway) simulations versus measured diurnal pattern of blood DCA concentrations in mice during a 24-h light-dark cycle of

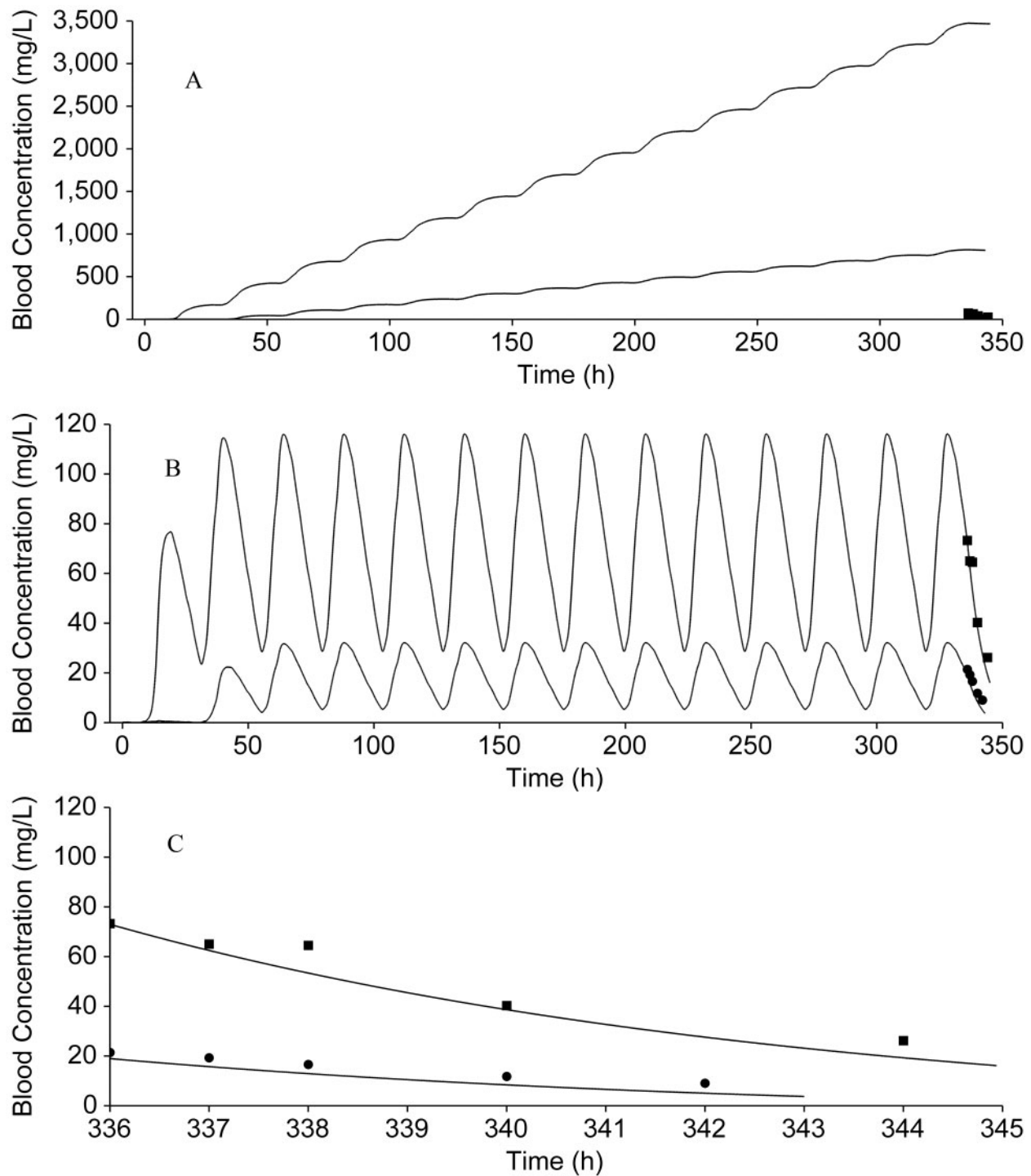


FIG. 4. Model simulations vs. measured concentrations of DCA in the blood of rats following exposure to 0.5 (●) or 2 (■) g/l DCA for 14 days in drinking water from model (a) without and (b,c) with noninhibitable, linear pathway 2. Measured values are presented as mean values ($n = 5$).

continued treatment with 2 g/l DCA in drinking water subsequent to 14 days treatment. The model does not exactly mimic the pattern of the data but captures the peak concentration of DCA and its rate of systemic clearance. Precise

agreement between the data and simulation is not expected, since the diurnal drinking patterns of the mice in this study likely varied from the prototype drinking water data utilized in model development. Different exposure patterns

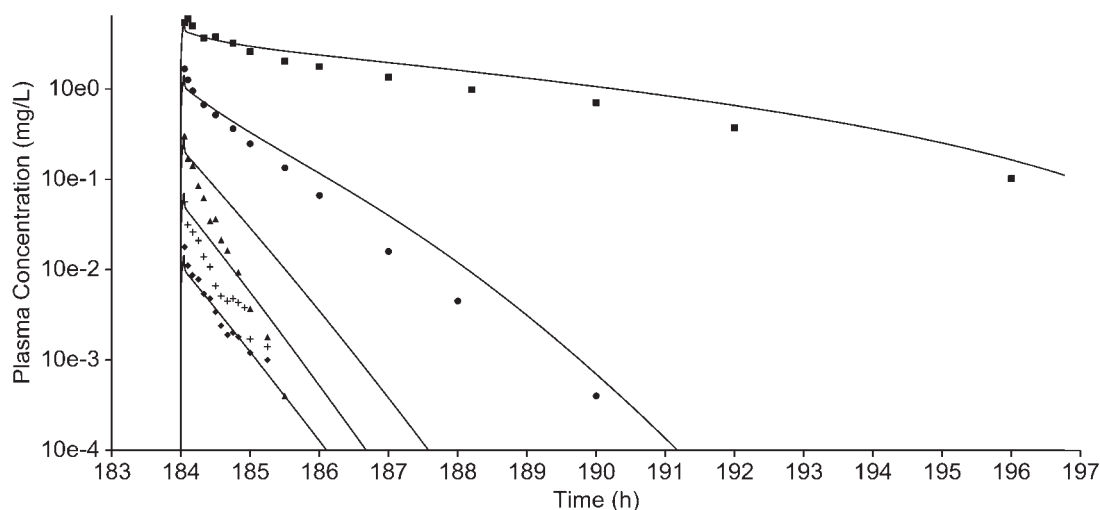


FIG. 5. Rat Model Validation. Simulations versus measured values for rats pretreated with 0.2 g/l DCA in drinking water for 7 days, followed by a 16-h recovery period and then exposed to 0.05 (◆), 0.25 (+), 1 (▲), 5 (●), or 20 (■) mg/kg DCA via iv injection. Measured values originally published by Saghir and Schultz (2002) and are presented as mean values ($n = 4-6$).

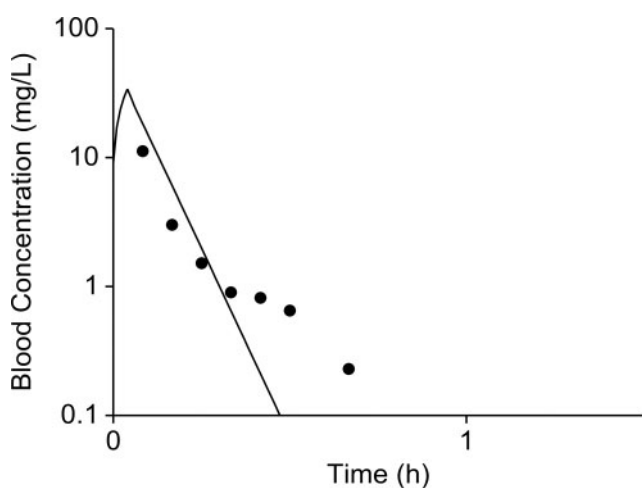


FIG. 6. Model simulations versus measured concentrations of DCA in the blood of naïve mice dosed with 20 (●) mg/kg DCA via iv injection. Measured values were originally published by Schultz *et al.* (2002) and are presented as mean values ($n = 3-8$).

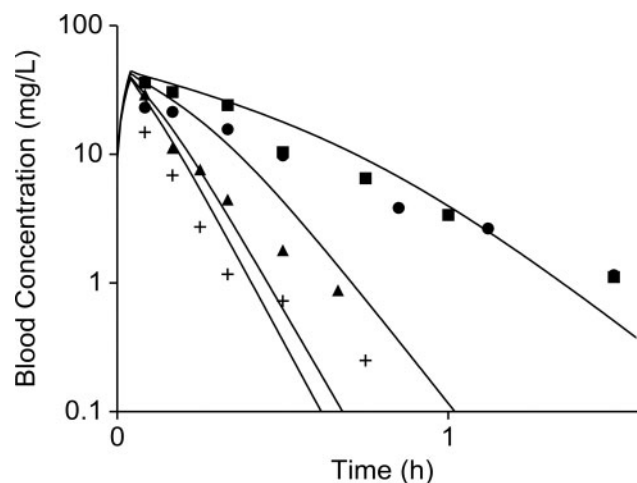


FIG. 7. Model simulations vs. measured values for mice pretreated with 2 g/l DCA in drinking water for 14 days, followed by a 6 (●), 16 (■), 36 (▲), or 48 (+) h recovery period and then dosed with 20 mg/kg DCA iv injection. Measured values were originally published by Schultz *et al.* (2002) and are presented as mean values ($n = 3-8$).

for drinking water (e.g., intermittent vs. continuous ingestion rates) would result in different patterns of DCA in the blood.

Mouse Model Validation

As a validation step, the model was run with no further alterations in parameter values, to predict blood concentrations of DCA in mice pretreated with 2 g/l DCA in drinking water for 14 days followed by a 48-h recovery period and dosed with 5 mg/kg DCA by iv infusion. The model tracked the first and next-to-last time points very well, while somewhat overpredicting the three middle time points (Fig. 9).

DCA Liver Concentration and Rate of Metabolism Predictions

The DCA model was calibrated based solely on blood and plasma pharmacokinetic data. Liver concentrations were predicted assuming a well-mixed liver model and a measured liver: blood partition coefficient in rats of 1.08. Predictions and measurements of DCA liver concentrations at various time points in rats and mice pretreated with 0.5 or 2 g/l DCA in drinking water are shown in Figures 10a and 10b. Mean liver concentrations are moderately overpredicted in the rat by a factor of approximately 2; however in the mouse model, predictions were not in close

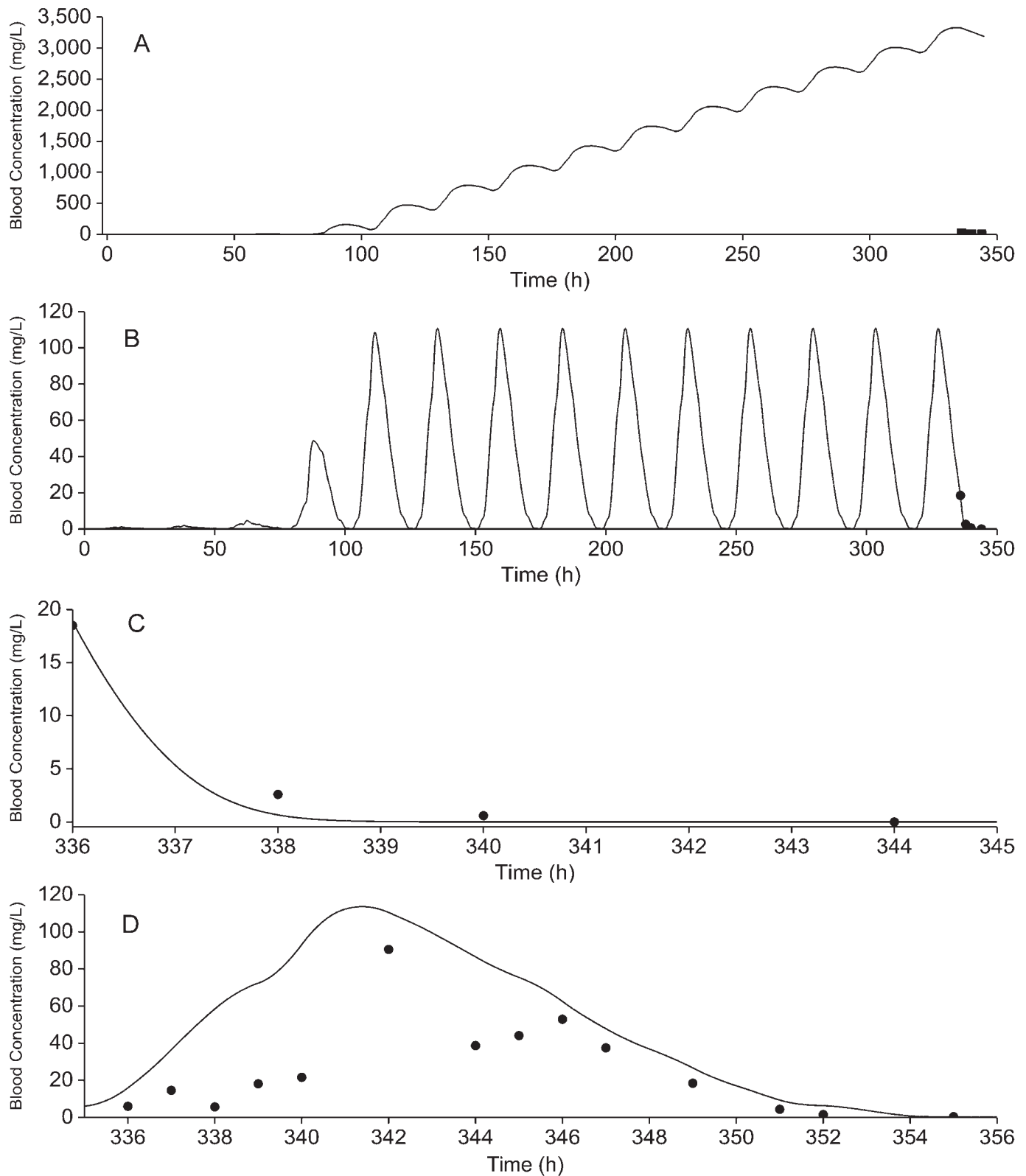


FIG. 8. Model simulations from model (a) without or (b,c) with noninhibitable, linear pathway 2 versus measured concentrations of DCA in the blood of mice previously treated for 14 days to 2.0 g/l DCA in their drinking water followed by removal of treated drinking water or (d) continued treatment with treated drinking water. (a, b, and c) Measured values are presented as mean values ($n = 5$). (d) Measure values were published by Schultz *et al.* (2002) and are presented as mean values ($n = 3$).

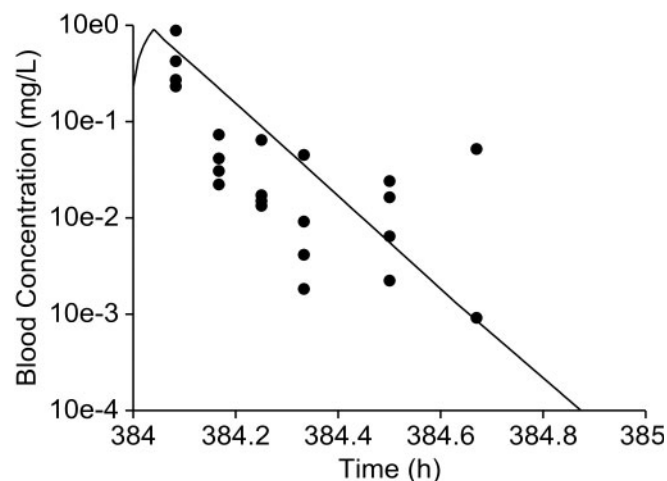


FIG. 9. Mouse Model Validation. Simulations versus measured values for mice pretreated with 2 g/l DCA in drinking water for 14 days, followed by a 48-h recovery period and then exposed to 5 (●) mg/kg DCA via iv injection. Measured values are presented as individual values ($n = 2-4$).

agreement with observation. The peak concentration of DCA and systemic clearance of DCA are overpredicted.

Comparison of the quantitative estimates of the maximum metabolism rate of DCA for both pathways described in this model, pathway 1, the saturable GSTzeta-mediated pathway, and pathway 2, the linear, GSTzeta-independent pathway, under conditions ranging from minimal to near maximal inhibition are given in Table 5. In naïve rodents (not pretreated with DCA), pathway 1 was predicted to account for 97% and 99.5% of the total metabolism in the rat and mouse, respectively. In contrast, with significant depletion of GSTzeta in the rodents by pretreatment with 2 g/l DCA for 14 days followed by a 16-h recovery period, pathway 1 was predicted to account for only 19% of total metabolism in the rat and 86% in the mouse. The estimated reduction in the rate of pathway 1 metabolism between the naïve and 2 g/l DCA pretreated rodents was 99% and 76% in the rat and mouse, respectively. The species difference is an indication of the impact of the higher estimated inhibition constant (k_d) in the rat than the mouse.

DISCUSSION

The estimated rate of initial inhibitable metabolism, $V_{\max 0C}$, was greater in the mouse than the rat. This ranking compares qualitatively with the work of Tong and colleagues (1998a), where the relative rate of DCA biotransformation by hepatic cytosol was significantly greater in the mouse compared to the rat. A $V_{\max 0C}$ of 40 mg/h/kg^{3/4} was used by Barton *et al.* (1999) to describe the initial maximal rate of DCA metabolism in naïve B6C3F1 mice. This is smaller than the value of 190 mg/h/kg^{3/4} used in the current study. However, Barton *et al.* also used a K_m value of 0.5 mg/l, which is smaller

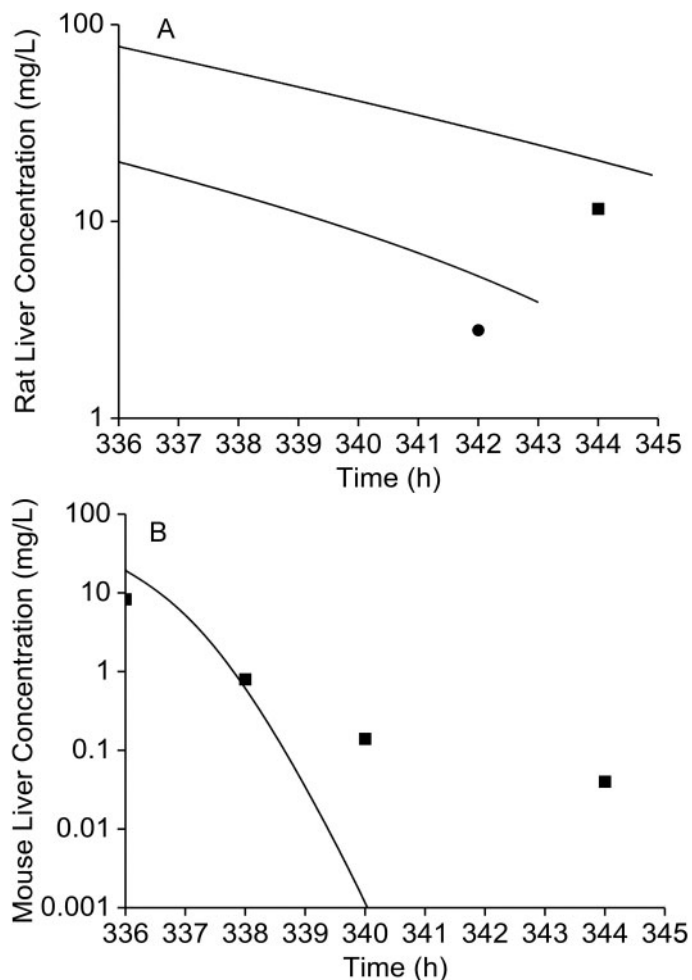


FIG. 10. Model simulations versus measured concentrations of DCA in the liver of (a) rats following exposure to 0.5 (●) or 2 (■) g/l DCA for 14 days in drinking water or (b) mice following exposure to 2 (■) g/l DCA for 14 days in drinking water.

TABLE 5
Model Predicted Maximum Rates of Metabolism of DCA

DCA water concentration (g/l)	Rate of metabolism (mg/h/kg ^{3/4})					
	Rat			Mouse		
	Pathway 1	Pathway 2	Total	Pathway 1	Pathway 2	Total
0.0	22.6	0.58	23.2	5.0	0.029	5.0
0.2	2.8	1.0	3.8	4.7	0.035	4.8
2.0	0.27	1.1	1.4	1.3	0.13	1.4

Note. Model predicted maximum rates of metabolism of DCA following an iv dose of 20 mg/kg in rodents pretreated with 0, 0.2, or 2 g/l DCA in drinking water for 2 weeks with a 16-h recovery period for GSTzeta-mediated (pathway 1) and nonspecific (pathway 2) pathways.

than the value of 10.6 mg/l used in the current study. The current study K_m estimate is taken from an *in vitro* liver cytosol enzyme-activity assay (Tong *et al.*, 1998a) which supports its use in this model. The estimated inhibition constant, k_d , was estimated to be larger in the rat than the mouse in this work. This compares qualitatively with the work of Tzeng and colleagues (2000) who found the rate constants for the DCA-dependent inactivation of GSTzeta in liver cytosol were also greater in the rat than the mouse.

A second proposed metabolic pathway representing a non-specific pathway of DCA metabolism was necessary to successfully predict DCA blood levels following 14 days of treatment with DCA in drinking water in both rats and mice. Without this second metabolic pathway, the model predicted accumulation of DCA in the blood, which was not observed. A linear description of the proposed nonspecific pathway was successful, using a first-order rate constant. The first-order rate constant was similar between species (1.15 for rat vs. 1.0 for mouse). This metabolic pathway may represent reductive chlorination of DCA to MCA or metabolic clearance by an additional GST isozyme. The results of this work show that the secondary metabolic pathway for DCA is important quantitatively in subchronic dosing scenarios such as the ones shown in Figures 4a and 8a. Under suicide inhibition conditions, the relative percentage of metabolism attributed to the secondary pathway increases, and this pathway becomes more critical for DCA elimination. The presence and further identification of this secondary pathway should be validated by further kinetic studies in the recently developed GSTzeta knockout rodents (Fernandez-Canon *et al.*, 2002).

A degradation rate (and by calculation, a corresponding zero-order resynthesis rate) for GSTzeta estimated from GSTzeta rat protein levels was used in this PBPK model assuming the rate of maximal GSTzeta-mediated metabolism (V_{max}) was proportional to the amount of GSTzeta enzyme present. The same rate was assumed in mice and worked adequately enough that major species differences in GSTzeta degradation would not be expected from the results of this modeling exercise. The fact that independently derived degradation rate and K_m values were able to adequately predict DCA blood levels in rats and mice lends confidence to the mechanistic nature of this PBPK model. An extensive database (five iv time courses with concentrations ranging from 0.05 to 20 mg/kg in pretreated rats) was available to validate the rat PBPK model blood concentration predictions with adequate success. There were less data available (one 5-mg/kg iv time course in pretreated mice) to validate the mouse model and, thus, less ability to judge the quality of the mouse PBPK model predictions.

More research is needed currently to understand the metabolic pathway and the stoichiometric yield of DCA from the metabolism of TCE. Substantial uncertainty exists in DCA blood levels following TCE dosing because of artifactual production of DCA (Ketcha *et al.*, 1996). Once such research is completed, the PBPK model developed in this work could be incorporated into an existing TCE model (e.g., Clewell *et al.*, 2000; Fisher,

2000) to more accurately predict DCA blood and liver dosimetry as a function of TCE dose. Reducing uncertainty in DCA dosimetry predictions would aid in reducing uncertainty in TCE dose-response assessment calculations (e.g., liver cancer slope factor), which are based, in part, on PBPK-model predicted dose metrics. A refined DCA submodel would also help elucidate the contribution of DCA to TCE-induced liver carcinogenicity in mice. Such reductions in uncertainty would have implications for TCE human health risk assessment.

Likewise, the ability of this DCA PBPK model to accommodate different drinking water exposure scenarios (length of exposure, concentration in drinking water, time of recovery) will increase its accuracy and ability to predict DCA blood and liver dose metrics that could be utilized in DCA risk assessment. Chronic treatment of B6C3F₁ mice with high concentrations of DCA in drinking water (2 g/l and higher) have resulted in hepatocellular carcinoma in approximately 95% of the animals (Bull *et al.*, 1990; DeAngelo *et al.*, 1991). Chronic treatment of male F344 rats with a mean daily concentration of 1.6 g/l DCA resulted in a 21% hepatocellular carcinoma incidence (DeAngelo *et al.*, 1996). Pharmacokinetic studies following 14 days treatment with concentrations of DCA of 2 g/l in drinking water in mice (Schultz *et al.*, 2002) and rats (Gonzalez-Leon *et al.*, 1997) show that the rate of DCA GSTzeta metabolism is significantly inhibited in both species. Thus, suicide inhibition of DCA GSTzeta-mediated metabolism is occurring at dose levels that clearly increase cancer incidence in rodent cancer bioassays. If the suicide inhibition occurs for the entire 2 years of the bioassay is an open question, since a recent study has shown age may act as a modifying factor, allowing older animals to adapt and be less sensitive to DCA suicide inhibition (Schultz *et al.*, 2002). If DCA is the proximate carcinogen, suicide inhibition would increase DCA potency. Alternatively, if a metabolite of DCA is the bad actor, suicide inhibition of DCA would be protective. More mechanistic information is necessary to fully understand the implications of suicide inhibition for DCA cancer risk assessment.

The current cancer dose response assessment for DCA included in the toxicological review of DCA (U.S. EPA, 2003) utilizes human equivalent doses derived from the doses used in animal bioassays. Once developed and validated, a human PBPK model for DCA could be used along with the rodent DCA model in this current work to predict internal dose metrics and refine the animal-to-human extrapolation in the DCA dose-response assessment in a similar manner to the draft 2001 TCE risk assessment (U.S. EPA, 2001). In addition, a PBPK model for DCA including suicide inhibition will allow for the inclusion of polymorphism-specific enzyme inhibition constants. Observed differences in polymorphic variants of recombinant human GSTZ-1 may indicate that subpopulations may differ in their susceptibility to toxicity from DCA (Tzeng *et al.*, 2000). The development of our PBPK model utilized data collected from three laboratories (Wright Patterson Air Force Base, Battelle Northwest, and University of Rochester Medical Center)

which lends confidence to its future application in both cancer and noncancer DCA dose-response assessment.

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